PB85-128189

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Oregon Health Sciences Univ., Portland

Nov 84

U.S. Department of Commerce
National Technical Information Service
NTIS

EPA-600/D-84-280
November 1984

PB85-128189

MODELS FOR EXCYSTATION

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TECHNICAL REPORT DATA (Please read Instructions on the reverse before completing)		
1. REPORT NO. EPA-600/D-84-280	2.	3. RECIPIENT'S ACCESSION NO. PB85 128189
4. TITLE AND SUBTITLE Models of Excystation		5. REPORT DATE November 1984
		6. PERFORMING ORGANIZATION CODE
7. AUTHOR(S) Ernest A. Meyer[1] and Frank W. Schaefer, III[2]		8. PERFORMING ORGANIZATION REPORT NO.
9. PERFORMING ORGANIZATION NAME AND ADDRESS [1]Dept. of Micro. & Immuno., Oregon Sciences University, Portland, OR 97201 [2]Microbiology Branch, TMD, HERL, U.S. EPA, Cinti., OH 45268		10. PROGRAM ELEMENT NO. AMBCIA
		11. CONTRACT/GRANT NO. (In-house)
12. SPONSORING AGENCY NAME AND ADDRESS Health Effects Research Laboratory Office of Research and Development U.S. Environmental Protection Agency Research Triangle Park, NC 27711		13. TYPE OF REPORT AND PERIOD COVERED Published Paper
		14. SPONSORING AGENCY CODE EPA-600/11
15. SUPPLEMENTARY NOTES		

16. ABSTRACT

Two methods are described for inducing G. lamblia to excyst. Both at times can be shown to yield trophozoites, although the success rate varies widely depending on the investigator and the cyst source. The excystation procedure for G. muris is much more satisfactory; however, results using it as a substitute or model for G. lamblia should be interpreted with great caution. It is suggested that all the factors involved in excystation have not been identified as yet. Several areas that appear to need rapid attention are the composition of the cyst wall, definition of factors in the incubation media that promote excystation, and determination of the presence or absence of endogenous enzymes associated with Giardia trophozoites. Also a study of the relationship of excystation to infectivity is needed badly. Presently excystation is only a measure of viability. The investigator interested in studying Giardia excystation should use these methods as a starting point for delineating the process even further.

17. KEY WORDS AND DOCUMENT ANALYSIS		
a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group
18. DISTRIBUTION STATEMENT Release to Public	19. SECURITY CLASS (This Report) Unclassified	21. NO. OF PAGES 29
	20. SECURITY CLASS (This page) Unclassified	22. PRICE

EPA Form 2220-1 (Rev. 4-77) PREVIOUS EDITION IS OBSOLETE

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MODELS FOR EXCYSTATION

Although the first recorded observation of Giardia trophozoites is generally attributed to Antony van Leeuwenhoek in 1681, the description of cysts of these organisms, and their relation to flagellated forms, was only made some two centuries later by Grassi (1879, 1881, 1888) and Perroncito (1887). The actual process of Giardia excystation has apparently only been seen and described in this century.

Simon in 1921 noted what appeared to be many immature Giardia trophozoites, and active movement within many cysts, in the freshly voided stool of an individual with giardiasis. This suggested to Simon that an exceptional thing had happened: that excystation had taken place in the digestive tract of this person. Hegner obtained a preserved sample of the fecal specimen in question from Simon, measured the free trophozoites therein, and reported in 1925 that they were much shorter than normal trophozoites. Although the process of Giardia excystation had neither been observed nor described at that time, Hegner agreed with Simon that excystation had probably taken place in the stool specimen, and that the small forms seen were newly excysted trophozoites.

Later (1927a,b,c), Hegner studied and sketched the excystation of Giardia cysts (from humans) which had been injected directly into the stomach of, then later removed from, rats. In this system, excystation did not occur in the stomach or duodenum, but in the jejunum or ileum. Hegner speculated that the factors responsible for excystation probably include: (1) a temperature of about 37 C, and (2) moisture. He also concluded (1927c) that host digestive juices are unnecessary in bringing about excystation.

Two years later, Deschiens (1929), in a study to determine whether Giardia cysts survive gastric passage, noted dead vegetative forms (trophozoites)

following exposure of cysts to human "digestive juice" and to aqueous hydrocloric acid.

In 1937, Armaghan placed cyst suspensions into rat stomach, duodenum, ileum, and caecum and found that the only rats subsequently infected with Giardia were those which had cysts administered in the stomach or duodenum.

For the next 40 years, the problem of identifying the factor(s) involved in Giardia excystation remained unstudied. In 1975, Lackie wrote: "...attempts to bring about excystation of Giardia lamblia in vitro were without success...the factors which initiate it (excystation) do not appear to have been identified."

Bingham (1979) and Bingham and Meyer (1979) reported the results of experiments which indicated that exposure of cysts to acid solutions at 37 C induces excystation of Giardia from humans, monkeys, dogs, rats, and mice. The process is pictured in Figure 1. Pepsin and salts were not necessary (Table 1).

Although hydrochloric acid was initially used, a variety of other inorganic acids also proved equally effective (Table 2).

The efficiency of excystation was shown to involve a relationship between the strength of the acid and the time of cyst exposure. Thus, as shown in Table 3, a maximal percentage of excystation was observed after a 5 to 15 minute exposure to pH 0.5, or a 10 to 30 minute exposure at pH 2.0.

Use of progressively higher pH in excystation showed that a decreased number of cysts was capable of excystation, and that progressively longer exposure times were required (Table 3).

Bingham et al (1979) also studied the effect on the excystation of Giardia of varying the temperature, both of the acid to which the cysts were exposed, and of the HSP-3 culture medium to which the treated cysts then were transferred. The results of these experiments are summarized in Table 4.

Bingham (1979) also studied the effect of the composition of the post-acid, incubation medium on excystation. He was able to show that significant levels of excystation were only induced in complete HSP-3 medium at pH 6.2 and 6.8 (Table 5). Furthermore, the percent excystation observed at pH 6.8 was significantly higher than at pH 6.2.

The components of HSP-3 medium include Hanks-Phytone broth, human serum, and tissue culture medium NCTC-135. In another experiment, Bingham (1979) compared the percentage excystation obtained after transferring acid-treated cysts into (a) Hanks-Phytone broth alone, (b) Hanks-Phytone broth plus human serum, and (c) complete HSP-3 medium. The results (Table 6) indicate that a significantly lower level of excystation took place in Hanks-Phytone broth alone, while the levels of excystation observed in HSP-3 and Hanks-Phytone with serum were not significantly different from each other. Tissue culture medium NCTC-135 thus did not appear to contribute anything to the excystation process that was not already present in the broth-serum mixture.

In 1979, Bingham and Meyer also employed the excystation procedure to determine the effect of temperature of storage on the viability of Giardia cysts obtained from their human donor. The method involved concentrating the cyst preparation promptly from a fresh stool, then determining the ability of the cysts to excyst after storage at various temperatures in tap water. Cysts stored at 8 C proved viable for 77 days or more, while those stored at 37 C never survived longer than 4 days. These workers also noted that a period of cyst maturation was apparently required before the Giardia used in their study reached full excystation potential. In all cyst suspensions they examined from fresh fecal specimens and stored at 8 or 21 C, low initial levels of excystation were observed. This period of low excystation lasted from 2 to 7 days and was followed by an increase in the level of excystation.

Subsequent excystation research suggests that additional factors may play a role in this process; the role of redox potential, for example, which was not measured in the above experiments, may well be of importance in the excystation of these organisms. The above experiments were conducted employing cysts from a single ~~door.~~ donor Subsequent observations suggest that the efficiency of excystation varies from donor to donor, and may also vary from time to time with cysts excreted by a single individual.

Rice and Schaefer (1981a) reported their experience with the Bingham-Meyer procedure of excysting Giardia from humans. They attempted, without success, to use the procedure on a routine basis, and subsequently developed a modified excystation procedure based on their G. muris excystation method (Schaefer et al., 1980) that regularly permitted them to excyst cysts from both symptomatic and asymptomatic donors.

The Rice-Schaefer procedure involves three steps: a low pH induction step, which utilizes three separate solutions, a wash step, and an incubation step, in which an excystment medium is employed. Briefly, the induction step involves the addition to the cyst preparation of hydrochloric acid (pH 2), Hanks balanced salt solution supplemented with L-cysteine hydrochloride, glutathione, and sodium bicarbonate, in that order. Immediately thereafter, the tube containing the cysts is capped and the contents mixed, then incubated for 30 minutes at 37 C. In the wash step which follows, the cysts are sedimented by centrifugation, and the supernatant discarded and replaced with excystment medium. The excystment medium consists of trypsin dissolved in Tyrode's solution, filtered, and then the pH adjusted to 8.0 with sodium bicarbonate solution. The cysts are again sedimented by centrifugation, the supernatant discarded and replaced with fresh excystment medium, and the resultant cyst suspension placed in depression slides. After the slides are

sealed and incubated (1 hour, 37 C), the percentage of cysts which had excysted is determined microscopically. This last manipulation is the incubation step.

Rice and Schaefer (1981a) reported the results of the use of this procedure in a total of 28 excystation trials, in 10 of which cysts were from 2 symptomatic donors, while 18 employed cysts from 3 asymptomatic donors (see Table 7). The lowest observed excystation percent (40 percent) involved cysts from an asymptomatic donor; the highest percentage (92 percent) involved cysts from both symptomatic and asymptomatic donors. The mean percentage excystation from the symptomatic donors (87 percent) was higher than that from the asymptomatic donors (70 percent). The lowest percentage of excystation these workers observed was 10 percent higher than that reported by Jarroll et al. (1981) using the Bingham-Meyer procedure.

In the same paper, Rice and Schaefer reported that repeated attempts, using the Bingham-Meyer procedure, consistently resulted in only a 2 to 3 percent excystment rate. These data indicate that the Rice-Schaefer method may be used successfully to excyst cysts from asymptomatic donors, but that in general higher percentages of excystation result, using this method, when cysts from symptomatic donors are employed.

Schaefer et al. (1980) also reported the results of excystation experiments employing Giardia muris cysts isolated from mouse feces. When they employed the Bingham-Meyer procedure, they obtained excystation efficiencies less than 5 percent. When they used the procedure they developed for G. muris excystation, the excystation percentages were consistently greater than 90 percent. Their G. muris excystation procedure, like that of Bingham-Meyer (1979) and Rice Schaefer (1981a) consists of the same steps: induction, wash and incubation. The main difference was that induction was affected by exposure of G. muris cysts to an induction solution consisting of 1 part reducing solution (Meyer, 1976) and 1 part 0.1 M sodium bicarbonate for 30 minutes at

37 C in a sealed test tube. When the induction components are mixed, carbon dioxide is evolved, the pH is 2, and the oxidation reduction potential is 120 mV. A gradual decline in excystation to 2 percent or less occurred as the pH and oxidation-reduction potential were changed to 7 and 57 mV, respectively. The induction step was followed by a wash and incubation step both carried out in 0.5% (w/v) trypsin-Tyrode's solution. This is the same solution used by Rice-Schaefer (1981a). The trypsin-Tyrode's is reported by these workers to be crude and somewhat tedious to make. The trypsin they used was an impure lyophilized extract of hog pancreas which does not completely dissolve after vigorous mixing for 30 minutes at ambient temperature. The undissolved trypsin was always removed by high speed centrifugation followed by positive pressure filtration. Schaefer et al. (1980) reported that trypsin-Tyrode's solution consistently produced better than 90% excystation after 30 minutes of incubation at 37 C in conjunction with the induction step. The G. muris excystation process is pictured in Figure 2. When the trypsin-Tyrode's solution was boiled, G. muris excystation declined to the 70th percentile indicating that the enzymatic activity of the solution is not crucial. Attempts by these workers to make trypsin-Tyrode's solution out of purified pancreatin resulted in little if any G. muris excystation. Similar results were obtained by Marchin (1982), when he used purified trypsin to make his trypsin-Tyrode's solution. Only when he substituted the crude for the purified trypsin was he able to affect G. muris excystations in the 90th percentile. Work by Sauch (1981) has recently shown that 0.5% (w/v) proteose peptone solution made in phosphate buffered saline at pH 7.2 can be substituted for trypsin-Tyrode's solution in the G. muris excystation procedure with equivalent results, provided the incubation period is extended from 30 to 45 minutes. In the case of G. lamblia cysts, Sauch was unable to get excystation when proteose peptone solution was substituted for trypsin-

Tyrode's solution. However, by replacing trypsin-Tyrode's solution with 1x Hanks balanced salt solution supplemented with phytone peptone, L-cysteine HCl, and sodium bicarbonate, she was able to get excystation of G. lamblia cysts. It is clear from the data that although enzymatic activity is not necessary for the incubation step of G. muris and G. lamblia excystation, it certainly seems to accentuate the process. Further, many factors important to the process remain to be identified.

Induction conditions for the Giardia species successfully excysted to date appear to mimic what is occurring in the stomach of the host. The pH of the mammalian stomach ranges between 1.5 and 2.5 (Prosser and Brown, 1961). Optimal induction pH for both G. lamblia and G. muris is around 2. Bergeim et al. (1945) reported that the oxidation-reduction potential of the rat stomach averages around + 150 mV, which approximates the optimal induction condition for G. lamblia (Rice and Schaefer, 1981a, b) and for G. muris (Schaefer et al., 1980). Carbon dioxide is found free in the lumen of both the stomach and small intestine. Calloway (1968) reported that the percent by volume of carbon dioxide in the stomach can range from 3.9 to 75.2, depending on the mammalian species. In the Rice-Schaefer procedure for G. lamblia excystation and the Schaefer et al. procedure for G. muris excystation bicarbonate is an induction ingredient which when mixed with the other induction ingredients evolves carbon dioxide gas. Physiological temperature is important for induction, as well, for G. lamblia (Bingham et al., 1979) and G. muris (Schaefer et al., 1981 a, b). Clearly there is nothing aberrant about G. lamblia and G. muris induction conditions with regard to carbon dioxide, oxidation-reduction potential, pH, and temperature when compared with these conditions known to occur in the mammalian stomach.

In mammals, as materials move from the stomach into the small intestine, the conditions encountered change radically with regard to pH and oxidation-

reduction potential. Magee (1962) reported that the pH of the duodenum, where Brunner glands are located, is usually between 7 and 8 in most species. Following meals, the proximal small intestine becomes acidic (Wormsley, 1971). The range reported was between 4 and 5. The Brunner gland area no doubt helps neutralize this acidity after meals. Fortran and Locklear (1966) stated that there is a pH gradient in the small intestine with the terminal sections becoming more neutral. Gradual neutralization of materials coming from the stomach occurs by the time the terminus of the small intestine is reached. In all Giardia excystation procedures reported to date there is a washing step after the induction step. The washing step appears similar to the neutralization that is occurring in the small intestine. While all Giardia incubation procedures used to date are static, they do provide a near neutral pH, nutrients, and physiological temperature. All these conditions are characteristic of the small intestine and mimic in vitro what is occurring in vivo.

Lackie (1975) reviewed the methods that have been used to activate infective stages of vertebrate endoparasites in vitro. Succinctly, the chemical and physical factors reported effective for protozoans included temperature, pH, reducing conditions, gases, enzymes, and bile. Generally a temperature around 37 C, like that for Giardia species was important in all cases except for coccidian forms in birds which possess body temperature between 39 and 41 C. The coccidian forms from birds have all adapted to the higher physiological temperature. Unlike Giardia species, other protozoan forms do not seem to require activation by exposure to low pH. However, they can tolerate exposure to acid conditions, because they normally pass through the stomach during the infection process. Reducing conditions were as important a trigger for the amoebas and coccidians as they were for the Giardia species. Likewise, carbon dioxide is as important for many of the coccidian

forms as it is for the Giardia species. Digestive enzymes were important for all the protozoans studied to date but the giardias. Generally the enzymes required were either trypsin or trypsin lipase. In no case has pepsin been shown as a requirement. Bile is of paramount importance only for the coccidians.

The requirement for a cyst maturation period of up to 7 days at 4 C reported by Bingham et al (1979) has been confirmed by Rice and Schaefer (1981a). Iwanczuk (1965) stated that for maximal infection to occur, G. muris cysts had to be stored in air at ambient temperature in the mouse feces between 2 and 24 hours. This requirement was not found for G. muris excystation by Schaefer et al. (1980). Giardia muris cysts isolated either after collection overnight or for two hours or less in water gave the same ninetieth percentile excystation result. For both fecal collection periods, the G. muris cysts were isolated as rapidly as possible to minimize exposure to the fecal material. Freshly purified G. muris cysts have been routinely used by these workers (unpublished data) for disinfection and infective dose studies. No difference between G. muris cysts a week or so old or those freshly isolated after only 2 hours collection has ever been noticed. Differences between the strains of G. muris may account for the contradictory findings.

The status of G. lamblia over the past 2 decades has changed from that of an innocuous gasrointestinal tract commensal to that of a pathogen affecting thousands of people. Waterborne transmission is the most frequently explained means of infection outbreaks (see Jakubowski and Hoff, 1979; Lippy, 1981). Consequently, there has been great interest in working with this organism. A major stumbling block impeding G. lamblia research has been the lack of an adequate laboratory experimental host through which to cycle the organism and the inability to get sufficient human donors to provide G. lamblia cyst containing stools. In most cases, asymptomatic carriers are

never diagnosed. While symptomatic cases are diagnosed, they are usually so distressed that they take curative medication just at the time they are identified. Due to the G. lamblia cyst scarcity, many workers in the field have begun to rely more on G. muris as a model (Roberts-Thomson et al., 1976) or a substitute for G. lamblia.

Although a great deal of information has been gained from the G. muris model research, it must be used with great caution. Over the past decade a number of striking differences have been documented between G. lamblia and G. muris. First, according to Filice (1952), the two organisms belong to distinct morphological groups. Giardia lamblia belongs to the duodenalis group which is characterized by the size range of 9 - 21 μm long by 5 - 15 μm wide and having claw hammer shaped median bodies. On the other hand, G. muris, which belongs to the muris group, is smaller with a size range of 9.2-16.9 μm long by 6.6-10.8 μm wide, and is characterized by rounded median bodies. In vitro culture of the trophozoite has shown another difference; G. lamblia can be serially subcultivated (Meyer, 1976; Visvesvara, 1980). Attempts to culture G. muris have not been successful to date. This immediately suggests a physiological difference between the two species. Transmission electron microscopy done on Giardia trophozoites of the muris type by Nemanic et al. (1979) has demonstrated the presence of endosymbionts in some of these organisms. This could be one reason for the inability to culture G. muris, since endosymbionts have never been reported in G. lamblia trophozoites.

As discussed above, cyst maturation has been documented for G. lamblia but not for G. muris excystation. Excystation of G. lamblia seems to occur with mineral acids, but these are not required for G. muris. Giardia muris consistently excysts in the 90th percentile; however, G. lamblia cysts, depending upon the cyst preparation and source, may excyst in a range anywhere from 0 to 90 percent. Empty cyst walls of G. muris are easily visualized by

phase contrast microscopy while G. lamblia cyst walls are not. Giardia cysts on isolation from fecal material show differences as well. Sauch (1983) has improved primary isolation methods for both G. lamblia and G. muris cysts. Her work has shown that the buoyant densities of cysts of the two species differ.

In summary, two methods have been described for inducing G. lamblia to excyst. Both at times can be shown to yield trophozoites, although the success rate varies widely depending on the investigator and the cyst source. The excystation procedure for G. muris is much more satisfactory; however, results using it as a substitute or model for G. lamblia should be interpreted with great caution. It is suggested that all the factors involved in excystation have not been identified as yet. Several areas that appear to need rapid attention are the composition of the cyst wall, definition of factors in the incubation media that promote excystation, and determination of the presence or absence of endogenous enzymes associated with Giardia trophozoites. Also a study of the relationship of excystation to infectivity is needed badly. Presently excystation is only a measure of viability. The investigator interested in studying Giardia excystation should use these methods as a starting point for delineating the process even further.

Table 1

Percentage Excystation of *Giardia* Cysts Exposed to Complete and Component-varied Synthetic Gastric Juice

Solution	What is this column	Mean[1] % excystation ± SEM
Complete (HCl + salts + pepsin)	1.6	25.8 ± 7.3
HCl + Salts	1.6	23.0 ± 7.0
HCl only	1.6	23.2 ± 7.0
Water (control)	6.8	0.1 ± 0.1
HSP-3 (control)	6.8	0.0 ± 0.0

[1]Means are derived from a total of 1191 to 1724 cysts which were counted in each solution.

Table 2

Percentage Excystation of Giardia Cysts Exposed to Inorganic Acids at pH 2.0 to 2.1

Acid	Mean[1] % excystation ± SEM
Water (control)	0.2 ± 0.2
HCl	17.4 ± 2.2
HNO_3	12.5 ± 3.0
$HClO_4$	15.5 ± 2.0
H_2SO_4	15.5 ± 2.6
H_3PO_4	19.4 ± 2.8

[1]Means are derived from a total of 2096 to 2392 cysts which were counted in each solution.

Table 3

Giardia Excystation with pH or Acid Solution and Time of Exposure Varied

	Exposure Time (Minutes)											
pH	5	10	15	20	30	60	90	120	180	240	MOET[1]	MO%E[2]
0.5	243[3]	256	231	..[4]	127	38	—	—	—	—	10	243
2.0	28	248	—	222	203	100	53	20	—	—	20	224
4.0	0	1	—	5	28	23	2	9	2	—	45	26
6.2	—	—	—	—	0	3	8	6	4	0	120	5

[1]Mean Optimum Exposure Time - Average of exposure times at which optimum percentages of excystation were not significantly different from each other at a given pH.

[2]Mean Optimum Percent Excystation - Average of optimum percentages of excystation which are not significantly different from each other at a given pH.

[3]Values represent percentages of excystation with reference to control tube (pH 2.0, 60 minutes) which is arbitrarily 100%. Underlined values represent optimum percentages of excystation at a given pH which are not significantly different from each other ($p > 0.01$).

[4]Excystation was not determined at these combinations.

Table 4

Excystation of *Giardia* Cysts Exposed to Temperature-Varied HCl (pH 2.0) and Transferred to Temperature-Varied HSP-3

Acid Temperature (°C)	HSP-3 temperature[1] (°C)		
	8	21	37
8	0	0.5	1.5
21	0	3.0	5.4
37	0	15.6	100.0

[1]Values represent percentage of excystation with reference to the control tube (HCl 37C, HSP-3 37C) which is arbitrarily 100%.

Table 5

Excystation of *Giardia* Exposed to HCl (pH 2.0) and Transferred into Various Solutions

Post-Acid Solution	pH	Percent Excystation[1]
Water	7.0	0.3
HCl	2.0	0.8
Saline (0.85 M)	7.0	0
PBS	7.0	1.1
HSP-3	0.5	0
	2.0	0
	4.0	0
	6.2	63.3
	6.8	100.0

[1]Percentages of excystation with reference to the control tube (pH 6.8 HSP-3) which is designated arbitrarily as 100%.

Table 6

Excystation of *Giardia* Exposed to HCl (pH 2.0) and Transferred into Component-Varied HSP-3 at pH 7.0

Post-Acid Solution	Mean[1] Percent Excystation ± SEM
Hanks-Phytone broth	13.1 ± 1.4
Hanks-Phytone broth + serum	42.7 ± 3.1
Complete HSP-3 medium	50.9 ± 4.0

[1]Means are derived from an average of 1205 cysts counted in each solution.

Table 7

Excystation of *G. lamblia* Cysts from Symptomatic and Asymptomatic Carriers

Donor	Batch	No. of Trials	Mean Percent Excystation[1] ± Standard Error	% Range
Symptomatic I		6	90 ± 2.2	79-95
Symptomatic II		4	83 ± 2.4	75-87
Asymptomatic I	A	4	69 ± 2.6	60-73
	B	3	56 ± 2.7	50-61
Asymptomatic II	A	3	42 ± 1.5	40-46
	B	5	92 ± 1.1	88-95
Asymptomatic III		3	77 ± 2.1	73-82

[1]Excystation percentages were based on counting 800 to 1,000 cysts in each trial.

Figure 1

Representative Steps in Giardia lamblia Excystation.

a. typical cyst

b.-f. sequential emergence of trophozoite(s) [arrows (1) indicate ventral adhesive discs of daughter trophozoites]

g. excystation completed, division of daughter trophozoites continuing

h. empty cyst

scale bar = 10 µm

(Bingham and Meyer, 1979)

FIG # 1

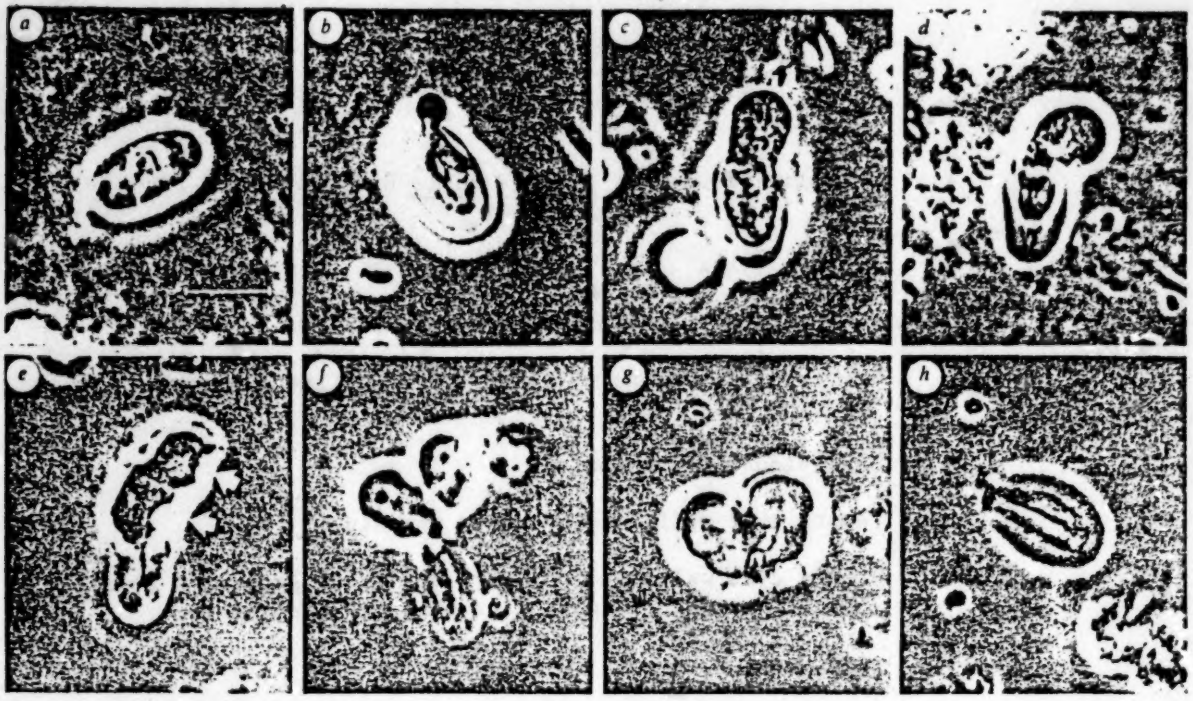


Figure 2

Sequential Stages of *Giardia muris* Excystation

a. intact cysts

b.-d. successive stages of trophozoite emergence

e. cytokinesis beginning, dart points to the place where sucking disk forms

f. individual trophozoites

g. empty cyst walls as seen from the side

h. empty cyst walls on end, dart points to scolloped polar opening through which the trophozoite emerged

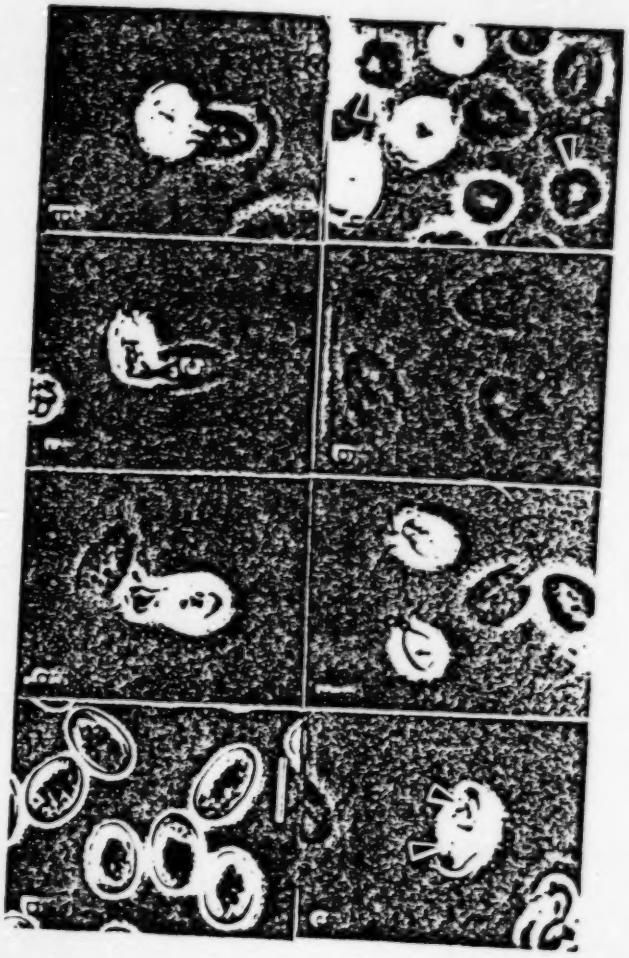
C = cyst wall

T = quadrinucleate trophozoite

t = binucleate trophozoite

scale bar = 10 µm

FIG #2



REFERENCES

Armaghan, V. 1937. Biological studies on the Giardia of rats. American Journal of Hygiene 26:236-258.

Bergheim, O., Kleinberg, J., and Kirch, E.R. 1945. Oxidation-reduction potentials of the contents of the gastrointestinal tract. Journal of Bacteriology 49:453-458.

Bingham, A.K. 1979. Studies on the excystation of Giardia. Master of Science Thesis, Oregon Health Sciences University.

Bingham, A.K. and Meyer, E.A. 1979. Giardia excystation can be induced in vitro in acidic solutions. Nature (London) 277:301-302.

Bingham, A.K., Jarroll, E.L., Meyer, E.A. and Radulescu, S. 1979. Induction of Giardia excystation and the effect of temperature on cyst viability as compared by eosin-exclusion and in vitro excystation. In Waterborne Transmission of Giardiasis. Proceedings of a Symposium. EPA-600/9-79-001. W. Jakubowski and J.C. Hoff, eds. pp. 217-229.

Calloway, D.H. 1968. Gas in the alimentary canal. Handbook of Physiology (Sect. 6), 5:2839-2859. American Physiological Society, Washington, D.C.

Deschiens, R. 1929. Chimisme gastrique et infections parasitaires du tube digestif. Annales de L'Institut Pasteur (Paris) 43:1353-1369.

Felice, F.P. 1952. Studies on the cytology and life history of a Giardia from the laboratory rat. University of California Publications in Zoology 57:53-146.

Fortran, J.S. and Locklear, T.W. 1966. Ionic constituents and osmolality of gastric and small intestinal fluids after eating. American Journal of Digestive Diseases 11:503-521.

Grassi, B. 1879. Intorno a speciali corpuscoli (Psorospermi) dell'uomo. Rendiconti, (R. Instituto Lombardo (Milano)) II (2):632.

Grassi, B. 1881. Intorno ad alcuni protisti endoparassitici ed appartenenti alle classi del Flagellati, Lobosi, Sporozoi e Ciliati. Atti della Societa Italiana di Scienze Naturali 24:135.

Grassi, B. 1888. Morfologia e sistematica di alcuni protozoi parassiti. Atti della Reale Accademia Nazionale dei Lincei, Rendiconti, (4 ser.) IF, (1 sem.) 5.

Hegner, R.W. 1925. Excystation in Giardia lamblia from man. American Journal of Hygiene 5:250-257.

Hegner, R. 1927a. Excystation and infection in the rat with Giardia lamblia from man. American Journal of Hygiene 7:433-447.

Hegner, R. 1927b. The viability of cysts of Giardia lamblia from man in the stomach of the rat. American Journal of Hygiene 7:782-785.

Hegner, R. 1927c. Excystation in vitro of human intestinal protozoa. Science 65:577-578.

Iwanczuk, I. 1965. Studies on the course of infection with Lamblia muris Bensen, 1908 in albino mice. Acta Parasitologica Polonica 13:227-258.

Jakubowski, W. and Hoff, J.C. 1979. Waterborne Transmission of Giardiasis. Proceedings of a Symposium. EPA-600/9-79-001.

Jarroll, E.L., Bingham, A.K., and Meyer, E.A. 1981. Effect of chlorine on Giardia lamblia cyst viability. Applied and Environmental Microbiology 41:483-487.

Lackie, A.M. 1975. The activation of infective stages of endoparasites of vertebrates. Biological Reviews 50:285-323.

Lippy, E.C. 1981. Waterborne giardiasis. Proceedings of the International Conference on Lake Management and Restoration, pp. 386-389. EPA-440/5-81-010.

Magee, D.F. 1962. Gastrointestinal Physiology. Charles C. Thomas, Springfield, p. 117.

Marchin, G.L. 1982. (Personal Communication).

Meyer, E.A. 1976. Giardia lamblia: isolation and axenic cultivation. Experimental Parasitology 39:101-105.

Nemanic, P.C., Owen, R.L., Stevens, D.P., and Mueller, J.C. 1979. Ultrastructural observations on giardiasis in a mouse model. II. Endosymbiosis and organelle distribution in Giardia muris and Giardia lamblia. Journal of Infectious Diseases 140:222-228.

Perroncito, E. 1887. Incapsulamento del Megastoma intestinale. Giornale della R. Accademia di Medicina Torino, Nos. 9 & 10. Transl. in Zentralblatt fuer Bakteriologie, Parasitenkunde, Infektionskrankheiten und Hygiene II:738.

Prosser, C.L., and Brown, Jr., F.A. 1961. Comparative Animal Physiology, 2nd ed. W.B. Saunders Co., Philadelphia. p. 112.

Rice, E.W. and Schaefer, III, F.W. 1981a. Improved in vitro excystation procedure for Giardia lamblia cysts. Journal of Clinical Microbiology 14:709-710.

Rice, E.W. and Schaefer, III, F.W. 1981b. An improved in vitro excystation procedure for Giardia lamblia cysts. Program and Abstracts of the 56th Annual Meeting of the American Society of Parasitology, Montreal, Abstract no. 150.

Roberts-Thomson, I.C., Stevens, D.P., Mahmoud, A.A.F., and Warren, K.S. 1976. Giardiasis in the mouse: an animal model. Gastroenterology 71:57-61.

Sauch, J.F. 1981. (Personal Communication).

Sauch, J.F. 1983. Improved primary isolation methods for Giardia cysts. 83rd Annual Meeting of the American Society for Microbiology, New Orleans, Abstract no. Q13.

Schaefer, III, F.W., Rice, E., and Hoff, J.C. 1980. In vitro excystation of Giardia muris cysts. 55th Annual Meeting of the American Society of Parasitologists, Berkeley, Abstract no. 90.

Simon, C.E. 1921. Giardia enterica, a parasitic intestinal flagellate of man. American Journal of Hygiene 1:440-491.

Visvesvara, G.S. 1980. Axenic growth of Giardia lamblia in Diamond's TPS-1 medium. Transactions of the Royal Society of Tropical Medicine and Hygiene 74:213-215.

Wormsley, G.S. 1971. Reactions to acid in health and disease. Gut 12:67-84.